## REMARKS

Reconsideration is respectfully requested in view of the foregoing amendments, the remarks which follow and the attached Rule 132 Declaration.

By this Amendment claims 32-33, 46-54 and 76-78 have been cancelled without prejudice or disclaimer.

Claims 7, 38, 55, 58 and 59 have been amended. Support for these amendments is to be found in the as-filed specification.

New claims 80-86, inclusive, have been added. These claims are supported in the as-filed specification.

Submitted herewith on a separate sheet of paper is the Abstract of the Application.

The Examiner has objected to scheme 6-7 at page 18 of the specification. This objection is traversed.

The reaction product between an alcohol and an epoxide should be R-O-$CH_2$-CH(OH)-R', wherein R is the residue of the alcohol and R' is the residue bonded to the epoxide. As further confirmation, Applicants submit a photocopy derived from *CHIMICA ORGANICA* Allinger et al. Zanichelli. (SEE ANNEX 1) (see reaction scheme 2).

Therefore in the aforementioned scheme 6 the term "spacer" is meant to comprise also the $CH_2$-CH(OH)- moiety. The above finds support at page 13, lines 7-9 of the specification where it is stated that the spacer may be, for example, selected from the group consisting of an **aliphatic** or araliphatic chain, **linear** o-branched , substituted by one or more groups selected from **hydroxyls**,...". Since the objection has been overcome, its withdrawal is solicited.

In the reaction product of scheme 18, the hydroxyl of the taxol was erroneously and inadvertently attached to the hydrogen. By amendment of the specification herein, the hydrogen has been deleted by striking through it.

The activation of hydroxyl in the formation of carbamates of urethane is a term which is universally accepted to indicate that the formation of carbamate or urethane bonds between a hydroxy group of a molecule and an amino group of another molecule is achieved by means of a well known activating agent, for example, those reported in the scheme in Annex 2, enclosed. In particular, the activating agent mentioned is 1,1-carbonyldiimidazole or N-N' disuccinimidyl carbonate as reported in Annex 3, enclosed herewith, the above activating agents are moreover mentioned as hydroxyl activating agents, at page 21, lines 1-3 of the specification. This objection having been overcome, it is respectfully requested that it be withdrawn.

The rejection of claims 46-48 and 76-78 has been rendered moot in view of their cancellation.

The Examiner has rejected claims 37-38, 55, 58-59, 63, 68, 70, 73 and 78 under 35 U.S.C. § 112, second paragraph, for indefiniteness. This rejection is respectfully traversed.

In claims 37-38, the wording "bond percentage" has been replaced with "substitution degree",

In claim 55, Applicants deleted the wording "stent" and a new claim 80, dependent from claim 55, has been added claiming specifically the stent as a medical device. Analogously, Applicants have deleted the wording "stent" from the claim 79 and drafted a new claim 81 claiming specifically that this process serves for preparing a stent.

Applicants deleted the word "thiourethane" from claim 58.

Applicants have previously amended "bounded" to read --bonded-- in claim 59 by their Preliminary Amendment of April 18, 2005.

Also in claim 59, Applicants have replaced the wording "acetyl" with --acetal--, and deleted "such as formaldehyde" and "such as bromine".

In view of the foregoing, the § 112 rejection has been overcome and should be withdrawn.

**Claims rejections under 35U.S.C. § 103(a)**

The Examiner has rejected claims 1-48 and 55-79 under § 103(a) as being obvious over Luo et al. in view of Sparer et al., Li et al., and Desai. This rejection is respectfully traversed.

To overcome the Examiner's rejection, Applicants enclose herewith a Rule 132 Declaration, wherein the *in vitro* data reported in Example 2 were transformed as a function of the taxol equivalent in order to make a comparison with the data reported by Luo et al.

First of all, from this Declaration it develops that the correct datum for taxol IC50 on MDA/MB/231 cells and reported in the table of example 2 in the specification of the instant application is not 0.35nM, but 0.35 **µ**M as it comes out from a careful reading of figure 2 wherein the ratios of IC50 of taxol vs. the compounds of the invention are reported in the form of a graph. In fact, the correct dimension for the IC50 cannot be nM, but must be µM.

In fact, 0.35 µM corresponds to 350nm consequently the aforementioned ratio is 350/2.58=**135, which coincides with the value reported in Figure 2.**

By applying the mathematical formulas reported in the Declaration at pages 3-6, the % molar derivatization degree or %substitution degree $_{\text{moles of taxol/moles of HA}}$ of the compounds of the invention tested in Example 2 were calculated. These calculations gave the following results.

HYTAD1p20 ester derivative of HA covalently bonded to paclitaxel with an esterification degree calculated as w/w of 16%, wherein the average molecular weight of

HA is 200,000 Da, corresponding therefore to a substitution degree expressed as % moles of taxol/mole of HA = **9.05%.** This means, therefore, that this product contains 9.05 mole of taxol/100 mole of HA

HYTAD2p20 ester derivative of HA covalently bonded to paclitaxel with an esterification degree of the carboxyl calculated as w/w of 22%, wherein the average molecular weight of HA is 39000 Da corresponding therefore to a substitution degree expressed as moles of taxol/mole of HA x 100 = **13.4%.**

HYTAD2p10 ester derivative of HA covalently bonded to paclitaxel with an esterification degree of the carboxyl calculated as w/w of 6.8%, wherein the average molecular weight of HA is 39000 Da, corresponding therefore to a substitution degree expressed as moles of taxol/mole of HA = **3.4%.**

If we now convert the values reported in the table of example 2 (corrected as regards the value for IC50 of paclitaxel as discussed previously) in order to determine the IC50 expressed in amounts of taxol equivalents ($IC50_{\text{taxol equivalents}}$) contained in the above three (3) tested compounds from the IC50 expressed as nM of tested product ($IC50_{\text{nM tested compound}}$) by applying the following mathematical formula:

$IC50_{\text{taxol equivalents}} = IC50_{\text{nM tested products}} \times \%\text{substitution degree}_{\text{moles of taxol/mole of HA,}}$

the following table is obtained:

| Cells lines | Taxol | HYTAD2p20 | HYTAD1p20 | HYTAD2p10 |
|---|---|---|---|---|
| MCF/7 | 3.5 nM | **0.11 nM** of taxol equivalent | **0.0022 nM** of taxol equivalent | **0.023 nM** of taxol equivalent |
| MDA/MB/231 | 0.35 **µM** | **0.34 nM** of taxol equivalent | | **8.16 nM** of taxol equivalent |
| MDA/MB/468 | 9.4 nM | | **0.016 nM** of taxol equivalent | |
| SKBR/3 | 0.23 nM | | | **0.0047 nM** of taxol equivalent |

It follows therefore that the compounds of the invention are, respectively, decidedly more effective than taxol on:

- MCF/7 cells, respectively,: 3.5/0.11 = **31.8 times** 3.5/0.0022= **1590** times 3.5/0.023 = **152.1** times

- MDA/MB/231, respectively: 350nM / 0.34nM = **1100** times , 350nM/8.16= **43 times**

- SKBR/3: 0.23/ 0.0047 = **48.9** times

**LUO ET AL**

Luo et al. disclose that it is known that HA is over expressed at sites of tumour attachment to the mesentery and provides a matrix that facilitates invasion. Several type of cellular HA receptors respond to HA as a signal and these include CD44 and RHAMM, the receptor for HA mediated cell motility. (See page 756, lines 21-30.)

In view of the above, the selectivity for cancerous cells could be markedly enhanced and overall dosages may be reduced by coupling antitumour agents to HA which can produce advantages in drug solubilization, stabilization, and localization.

Consequently, Luo et al. studied the antitumour activity of the conjugates HA-Taxol linked to each other by means of adipic hydrazide (defined by Luo et al. as HA-ADH-taxol or HA-taxol).

Table 2 at page 760, right-hand column, reports different bioconjugate HA-ADH-Taxol, having taxol loadings ranging from 1.2% to 15 % by using different ADH loadings (9-18 and 45%) and this taxol loading being a molar ratio, as is the corresponding starting ADH loading, which was calculated by integration of the ADH methylene signals (see page 760, right-hand column, lines 24-30).

From the same table it can be seen that by increasing the taxol loadings, the solubility diminishes. In fact, the HA-taxol with a taxol loading of 14.9%, is partially soluble in water, whereas a taxol loading containing 15% is completely water insoluble.

The cytotoxicity of these HA-taxol compounds was determined and the results thus obtained are presented in table 3 where it can be seen that for the least modified HA (9% ADH modification), higher toxicity was observed as the taxol loading increased. However, the cytoxocity of the highly modified HA (45%) actually decreased at the

highest taxol loading (15%) (see page 762, right-hand column, line 4 from the bottom to page 762, left-hand column, line 3), since high loadings of taxol decreased the solubility of the HA taxol–conjugate by masking the HA receptor recognition causing the aggregation of the polymeric conjugate, thus limiting the toxicity of the conjugate relative to the free drug. (See page 762 lines 3-7.)

For the above reasons although the HA-Taxol with the highest cytotoxicity is preparation or product 7 having a taxol loading of 14.9%, this product, however, is partially water soluble. For the subsequent study, which is addressed to compare the cytotoxicity of the conjugate HA-taxol with that of free taxol, the conjugate containing 5% of taxol was selected for the study since it is **the most effective and contemporaneously water soluble.**

Figure 8 summarizes the results of the study by Luo et al. From Figure 8 which reports in ordinates the cell viability (%) and in abscissae the concentration of taxol equivalent (μg/ml), it results that the IC50 of the HA-taxol containing a taxol loading of 5% is 0.05 μg/ml versus a IC50 for taxol, as such, of about 0.11 μg/ml, thus indicating that this conjugate is at most **twice** as effective as taxol, as such.

Apart from **the standard** error **range which is very great**, with the consequence that the data reported in this graph are **not** statistically significant, as admitted by the authors, the Luo et al. conjugates show a cytoxicity **comparable** to that of free taxol. Therefore, the above data in Luo et al. is decidedly poorer than the statistically significant data obtained with the compound of the claimed invention which are from **30 to 1500** times more cytotoxic and, therefore, more powerful than taxol, per se. In particular, those with a low taxol loading of 3.4% (which are more similar to the taxol loading analysed by Luo (5%)) were found to be **152, 43** and about **49** times more cytotoxic and, therefore, more effective than free taxol.

In view of the foregoing, although Luo et al. teach that in addition to the advantages with respect to drug stabilization, solubilization, and controlled release of the conjugated drug, the HA-Taxol conjugates had the advantage of the presence of hyaluronic acid as the carrier, which is immunoneutral, and has viscoelsticity that makes

it an excellent joint lubricant, is biocompatible and has been used as vehicle, **however, the experimental results reported by Luo et al. are poor and would not be viewed as very promising by one of ordinary skill in the art**.

Therefore, one of ordinary skill in the art in carrying out a serious analysis of the results achieved by Luo et al., and also considering that they are **not** statistically significant, would in **no way** be validly taught that hyaluronic acid conjugates with paclitaxel showed **real advantages** when compared to paclitaxel as such. Any advantages these compounds showed were not significant, **but at most** attributable to the improved solubility of these conjugates when compared to the solubility of paclitaxel.

It is submitted that one of ordinary skill in the art from a reading of Luo et al. would not have been motivated to believe that different HA- taxol conjugates, with comparable taxol loadings, would have had such a ***decidedly higher cytotoxic action***, namely, **from 43 to 152 times higher**, than that of paclitaxel as such, thus implying a **synergistic therapeutic** role of the hyaluronic acid in the conjugated compound.

It follows from the foregoing that Luo et al., besides not disclosing the specific taxol conjugates, as claimed in claim 1 of the instant application, were also far removed from suggesting their unexpectedly excellent antitumoral activity.

Luo et al. deficiencies can **in** no **way** be overcome by the disclosure of Sparer et al. which discloses GAG conjugates with different active ingredients from paclitaxel, namely, the antibiotic chloramphenicol and cysteine.

The cloramphenicol is bonded to GAG by means of and ester linked directly to the HA backbone or indirectly through the intermediate linking group alanine (see figure 1, page 112) by an ester bond between alanine and chlorpamphenicol and an amide bond between alanine and HA.

The cysteine is bonded to a GAG directly by means of an amide bond (see figure 2, page 112).

The conclusion of the Sparer et al. study is that in contrast to the above ester complexes which were released quickly (rates approximating first order kinetic hydrolysis of the ester bond), the release rate from the amide complexes was much lower and gave a constant rate of release. (See page 117, lines 15-19.)

In the Sparer et al. reference, however, there is nothing said to suggest a possible involvement of the GAG molecule and, in particular, that of HA, in increasing the therapeutic activity of the examined active ingredients, cysteine and chloramphenicol. The disclosure of Sparer et al. is decidedly remote from teaching or suggesting the presently claimed conjugate which contains an active ingredient **having no bearing or connection at all** with those encompassed by the Sparer et al. disclosure.

It follows from the foregoing that Sparer when combined with Luo et al. would be totally unable to suggest the presently claimed paclitaxel conjugates and that they had antitumoral effects **from 30 to 1500 times greater than the antitumoral effect of paclitaxel.**

The Luo et al. and Sparer et al. deficiencies can **in no way** be overcome by Li. In fact, this reference discloses **different** conjugated taxol from those claimed herein, since HA in the Li conjugate is replaced **by a polyaminoacid or a polyethylenglycol (see the abstract),** namely, chemical compounds which are completely different from HA, and having chemical and pharmaceutical activities which most decidedly are not comparable with those of HA.

The same consideration can be made when analyzing the Desai reference which discloses a polymeric drug delivery system in which the drug is bound to a water soluble polymer to provide a form of soluble drug delivery, especially when the drug is by itself water insoluble. In particular, the drug taxol is covalently bound to polyethylene glycols (see the Abstract).

It is respectfully submitted that in view of the foregoing, the claims distinguish over the combination of references employed by the Examiner. Accordingly, the § 103(a) rejection has been overcome and should be withdrawn.

The issuance of a Notice of Allowance is respectfully solicited.

Please charge any fees which may be due and which have not been submitted herewith to our Deposit Account No. 01-0035.

Respectfully submitted,

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